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TITLE: MT 2A Phosphorylation by PKC Mu/PKD Influences Chemosensitivity to
Cisplatin in Prostate Cancer

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14. ABSTRACT The metallothioneins (MT) are a family of small molecular weight trace metal and free radical scavenging proteins well established to play a role in resistance to chemotherapy and radiation in human cancer. MT gene expression is up regulated in response to the presence of heavy metal ions such as zinc. We have previously established a physiological in vitro cell line model of MT induction using Zn, which is significantly associated with resistance to cisplatin chemotherapy in PC. In this report, our data demonstrates that treatment with TPEN, a Zn specific chelator, significantly improves sensitivity to cisplatin in prostate cancer cells.					
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SECOND ANNUAL REPORT

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Introduction

Since submission of first annual report in November, 2005, the PI has moved to University of Massachusetts, Worcester, MA from University of Nebraska, Omaha, NE in March of 2006. Because we are in the process of transferring the grant to a new institution we have not accomplished our stated goals for the second year. Herein, we report on the progress made in the first three months of the second year.

Our preliminary studies had demonstrated that kinase domain of Protein kinase D1, a novel serine threonine kinase, interacts with Metallothionein 2A (MT 2A), which belongs to the family of small molecular weight proteins called metallothioneins (MT) that scavenge trace metals and free radicals and are associated with resistance to chemotherapy and radiation in human cancers¹. Our studies further demonstrated the C4-2 prostate cancer cells that express higher levels of MT compared to its parental LNCaP cells² are selectively more resistant to trace metal containing chemotherapy agent cisplatin compared to LNCaP cells. Our *in vitro* experiments also demonstrated that MT 2A was phosphorylated by PKD1. Therefore we hypothesized ““Alteration in MT 2A expression influences chemoresistance to cisplatin in prostate cancer. PKC Mu/PKD kinase activity influences sensitivity to cisplatin by MT 2A phosphorylation in prostate cancer. The expression of MT 2A is quantitatively increased in progressive human prostate cancer””.

We proposed to establish the stated hypothesis through 3 aims.

Aim 1. To determine that alteration in MT 2A expression influences resistance to cisplatin in prostate cancer.

Aim 2. Inhibition of PKC Mu/PKD kinase activity and its influences on chemoresistance in prostate cancer cells by modulating the phosphorylation of MT 2A.

Aim 3. To quantify and qualitatively evaluate MT 2A protein expression in progressive human prostate cancer.

Body

Task 1: *To determine that alteration in MT 2A expression influences resistance to cisplatin in prostate cancer.*

1a: Effect of MT 2A over expression influences resistance to cisplatin in prostate cancer

Experiment 1: Cloning and Expression of MT 2A protein in LNCaP prostate cancer and MT 2A null CHO cells. As previously stated in our first annual report we continued our efforts to develop stable LNCaP and CHO cell lines transfected with His tag labeled MT 2A cDNA. In addition, we also cloned DsRed fluorescent tagged MT 2A in LNCaP cells. We found that compared to His tag labeled MT 2A transfected cells, the DsRed tagged clones expressed high levels of exogenous MT 2A and are useful in studying subcellular localization as well (Fig 1).

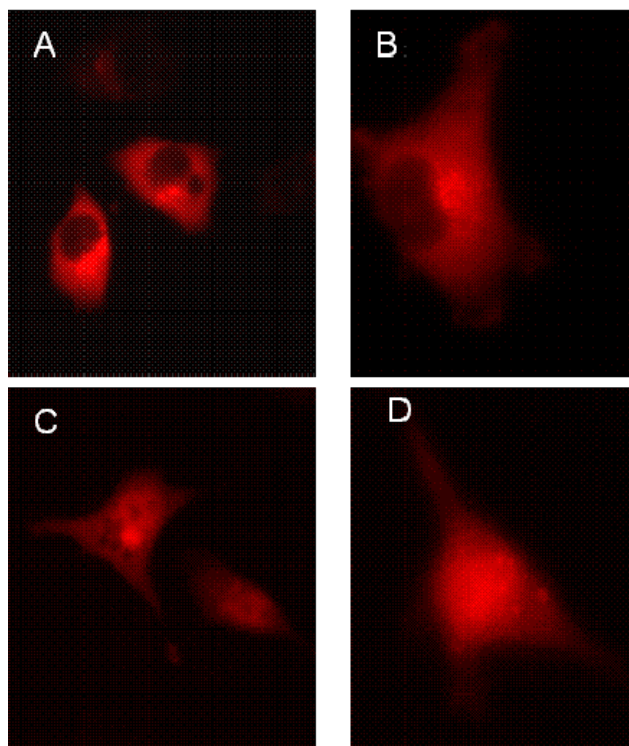


Figure 1; LNCaP prostate cancer cells transfected with DsRed fused MT 2A. Figures A and B demonstrate cytoplasmic localization of MT 2A and figured C and D demonstrate nuclear localization of MT 2A follow stimulation with either PKC activator phorbol ester (Fig 1C) or PKD1 selective activator Bryostatin1 (Fig 1D).

1b: Down regulation of MT2A by RNAi leads to increased sensitivity to cisplatin.

We reported in our first annual report that we were attempting to down regulate MT 2A using RNAi. Because of very low levels of endogenous MT in cells in unstimulated cells we were unable to demonstrate down regulation of MT 2A by RNAi. Therefore, we used an alternate model of MT induction by Zn followed by inhibition of MT induction by Zn specific chelator TPEN to study the effect of MT on resistance to cisplatin in prostate

cancer (Fig 2). Our results demonstrate that treatment of TPEN significantly improves sensitivity to cisplatin in prostate cancer.

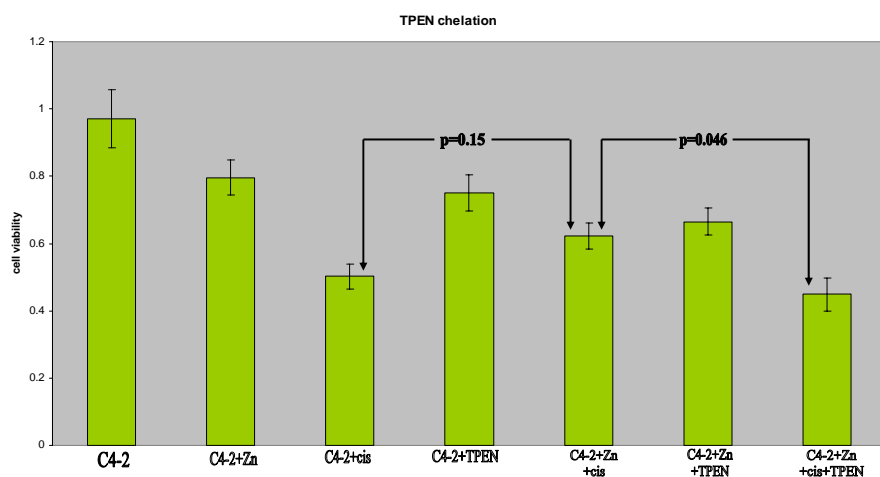


Figure 2: C4-2 prostate cancer cells were plated at a concentration of 3000 cells/ well in 96 well plates, treated with equimolar concentrations (150 μ M) of zinc, followed by treatment with TPEN at 24 hours and 10 μ M of cisplatin, which we had established as the IC50 concentration

in our cell line model. Viability assays were performed at 72 hours using non radioactive cell proliferation assays (Promega, Madison, WI). Experiments were performed in triplicate, and means were compared by 2-way ANOVA models. P values less than 0.05 were considered significant.

Key Research Accomplishments

We have established a stable DsRed fused to MT 2A cell lines, which would facilitate further studies on MT 2A and prostate cancer. We also recognized that down regulation of MT 2A by RNAi may not be a feasible experimental model because of very low levels of endogenous MT in prostate cancer cells. Therefore, we used an alternate model of inhibiting Zn induced MT

expression by Zn specific chelator TPEN, which demonstrated that TPEN treatment significantly increased sensitivity to cisplatin treatment in prostate cancer cells.

Reportable Outcomes

1. Tony Mammen, Samuel Sterrett, Cheng Du and K.C. Balaji; Treatment with Zinc Specific Chelator TPEN reverse An induced Resistance to Cisplatin in Prostate Cancer; Sante Fe, New Mexico, Poster #10, 10/06

Conclusions

MT expression is associated with resistance to cisplatin chemotherapy in human prostate cancer cells.

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2. Rao, P. S., Jaggi, M., Smith, D. J., Hemstreet, G. P. & Balaji, K. C. Metallothionein 2A interacts with the kinase domain of PKC μ in prostate cancer. *Biochem Biophys Res Commun* 310, 1032-8 (2003).